Maitake (Grifola frondosa) Improve Glucose Tolerance of Experimental Diabetic Rats

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Summary We have previously reported that rats with diabetes induced by injecting streptozotocin into neonates showed remarkably lower blood glucose, urine volume, and glucosuria after administration of Maitake (Grifola frondosa). In the present study, we investigated the effects of Maitake on insulin concentration, organ weight, serum composition, and islets of Langerhans in streptozotocin-induced diabetic rats using the same method. The diabetic rats were produced by injecting 80 mg/kg B.W. streptozotocin into 2-d-old neonates. From the age of 9 wk, the rats were given experimental diets for 100 d. The diabetes and control groups were given either diets containing 20% Maitake (DM and CM groups) or control diets (D and C groups). During administration of the experimental diets, we measured body weight, food intake, amount of feces, and serum insulin concentration at glucose loading. The glucose tolerance test was performed at the 10th week after the start of the experimental diets. The D group had an initial fasting blood glucose of 225±49 mg/dL, and a maximum blood glucose of 419±55 mg/dL at 60 min. In the DM group, however, the initial fasting blood glucose was 170±23 mg/dL, and the maximum blood glucose was 250±41 mg/dL at 15 min. Both values were markedly lower than those in the D group (p<0.05). The insulin concentration at 15 min. after glucose loading in the DM group was 41±16 µU/mL, which was significantly higher than that in the D group (15±7 µU/mL) (p<0.05). After the 100-d experimental period, blood samples were collected. The fructosamine level was significantly lower in the DM group (15±21 mmol/L) than in the D group (18±5±13 mmol/L). The concentration of 1.5-A.G. (1.5-anhydro glucitol) was significantly higher in the DM group (9.33±2.42 µg/mL) than in the D group (1.33±0.52 µg/mL). Observation of insulin antibody stain in the Langerhans cells of the pancreas using ABC method showed a decrease insulin antibody stain in the D group. The cells of the DM group were stained more darkly than those of the D group. From these results, we postulated that the bioactive substances present in Maitake can ameliorate the symptoms of diabetes.

Key Words Grifola frondosa (Maitake), serum composition, islets of Langerhans, streptozotocin-induced diabetic rats

The recent years have witnessed the great development of functional foods. One such food is the fruit body of mushrooms which previous research has shown to possess unique properties (1–3). For example, Grifola frondosa (Maitake) is a fungus belonging to Basidiomycetes, Aphyllophorales, Polypolaceae, and the fruit body of the fungi belonging to Basidiomycetes has been reported to contain useful bioactive polysaccharides (3–5). The antitumor activity of Maitake extracts is known to be due to β-1,3-glucans (6). Maitake also possesses lectin activity and antimutagenic, weight-reducing, cathartic, and geriatric disease-preventing effects in addition to blood pressure-lowering activity (7). We have also found that Maitake water extract can inhibit adipocyte conversion (8). What remains to be explained are the mechanisms of these effects.

Diabetes has been increasing continuously in Japan. The number of diabetes patients in 10 years may reach 10,800,000 (9). If hyperglycemia conditions persist, diabetic complications can arise. Thus, the prevention of hyperglycemic conditions is of top priority.

A recent study found blood glucose lowering activity in Ganoderma lucidum (Mannentake), one of the Basidiomycetes (10, 11). Kubo et al. reported this phenomenon for Maitake mushrooms using mice (12). We have also reported previously that Maitake could lower blood glucose level and sugar excreted in the urine of diabetic rats (13). We now needed to confirm whether blood glucose tolerance could be improved due to increased insulin secretion after administration of Maitake.

This study was undertaken to examine the effect of dried Maitake mushroom powder on the serum and Langerhans cells in the pancreas of diabetic rats.
MATERIALS AND METHODS

Preparation of diabetic rats and breeding methods. We used the method of Weir et al. to induce diabetes in rats (14).

Virgin albino Wistar rats weighing approximately 160 g were obtained commercially (from Kyudo, Kumamoto, Japan) and were fed commercial pelleted chow (CE-2 from CLEA Japan, Tokyo, Japan) until they weighed about 220 g. They were allowed to mate with males of the same strain. After confinement, diabetes was induced in the 2-d-old neonates by administration of streptozotocin dissolved in 0.9% physiological saline and adjusted to pH 4.5 with 0.1 M citrate buffer. This was prepared immediately before injection.

Two groups of neonates were raised: the first group received 80 mg/kg B. W. i.p. streptozotocin at 2 d after birth, while the second group received only citrate buffer at 2 d after birth.

All the rats (diabetic rats and control rats) were weaned 28 d after birth and fed commercial pelleted chow until 9 wk of age after which they were given experimental diets. The plasma glucose concentrations of diabetic rats induced by this method remained at nearly normal levels until 6 wk of age (14). Therefore the rats were administered experimental diets from 9 wk of age. The rats were divided into four dietary groups. The diabetic groups of 6 and 7 rats were given diets containing 20% Maitake powder (DM) or control diets (D) ad libitum throughout the experiments, respectively. In the same way, the control groups of 6 and 5 rats were given diets containing 20% Maitake powder (CM) or control diets (C) ad libitum, respectively. The experimental diets were given for 100 d. The rats were housed in individual mesh stainless steel cages in a room with controlled temperature (23 ± 1°C) and humidity (55 ± 10%) with a 12 h light-dark cycle. Water was supplied ad libitum throughout the experiments.

Experimental diets. The compositions of the experimental diets are given in Table 1. Their caloric contents were 4.38 and 4.57 kcal/g, respectively. The protein source was casein but for the DM and CM groups, its volume was adjusted in accordance with the Maitake protein content. The composition of other nutrients was not changed.

Body weight, amount of food intake and feces. The body weight was measured everyday during the administration periods of the experimental diets. The amounts of food intake and feces dried for 24 h were measured at the 12th week from administration of the experimental diets.

Glucose tolerance test and plasma insulin concentration. The glucose tolerance test was performed before administration of the experimental diets (8 wk old). The blood glucose was measured at 0, 15, 30, 60 and 120 min after injection of 1 g/kg B.W.i.p. glucose after 6 h fasting. The glucose tolerance test was performed again at the 10th week after administration of the experimental diets had begun. The concentrations of blood glucose were determined by glucose oxidase methods with a kit (Glucose C-II-test, Wako Pure Chemical Industries, Osaka, Japan). The concentrations of plasma insulin were determined by the Biotrak rInsulin enzymeimmunoassay system (Amersham Pharmacia Biotrak, UK). Plasma insulin concentration was measured before and at 15 min after glucose loading (10th week of experimental diets).

Anatomy of rats and preparation of organ sections. After the rats had been given the experimental diets for 100 d, they were starved overnight and vivisected under pentobarbital anesthetization. Blood from the heart was collected. The pancreases were extirpated and a piece was fixed in 10% formaldehyde neutral buffer solution. The fixed material was embedded in paraffin using an automatic embedding system (Shiraimatsu, Osaka, Japan) and then was cut in 4–5 μm-thick sections with a microtome. In order to observe insulin secretion in the islets of Langerhans of the pancreases, insulin was stained using the avidin-biotin-peroxidase complex (ABC) method (15). The nucleus was stained with hematoxylin and eosin.

The protocol of research using the animals was carried out according to the guidelines for breeding and

<table>
<thead>
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<th>Table 1. Composition of experimental diets (%).</th>
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<td>Choline-Cl</td>
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<td>Cellulose powder</td>
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<td>Maitake</td>
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<td>Energy (kcal/g)</td>
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1) Mineral mixture was prepared according to the AIN-76 formulation.
2) Vitamin mixture was prepared according to the AIN-76 formulation.

| Table 2. Contents of some components in Maitake used in this experiment. |
|-----------------------------|---------------|
| Moisture        | 7.5 (%)       |
| Protein         | 20.4          |
| Fat             | 2.3           |
| Carbohydrate    |               |
| Dietary fiber   | 42.5          |
| (β-Glucan)      | 23.5          |
| Non-fibrous     | 22            |
| Ash             | 5.3           |

Dietary fibers (by the enzyme and weight method) contained about 13.7% of fiber that was analyzed by the method of acid and alkali hydrolysis. β-Glucan, analyzed by the enzyme method, contained not only (1,3)-(1,6)-β-glucans but also cellulose, heteroglucans and other glucans.

Anatomy of rats and preparation of organ sections. After the rats had been given the experimental diets for 100 d, they were starved overnight and vivisected under pentobarbital anesthetization. Blood from the heart was collected. The pancreases were extirpated and a piece was fixed in 10% formaldehyde neutral buffer solution. The fixed material was embedded in paraffin using an automatic embedding system (Shiraimatsu, Osaka, Japan) and then was cut in 4–5 μm-thick sections with a microtome. In order to observe insulin secretion in the islets of Langerhans of the pancreases, insulin was stained using the avidin-biotin-peroxidase complex (ABC) method (15). The nucleus was stained with hematoxylin and eosin.

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Effects of Administration of *Grifola frondosa* on Diabetic Rats

Fig. 1. Changes in rat body weight during feeding with diet containing Maitake. The values are expressed as the mean±SD. a: significantly different for D in comparison with C and for DM in comparison with CM by Scheffe’s test (p<0.05). b: significantly different for CM in comparison with C and for DM in comparison with D by Scheffe’s test (p<0.05). c: significantly different for DM in comparison with C by Scheffe’s test (p<0.05).

safekeeping of laboratory animals (16).

Statistical methods. All statistical analyses were performed using two-way analysis of variance (two-way ANOVA) followed by post hoc Scheffe’s test to determine if the results were significantly different. The Stat View J 4.02 statistical analysis program (Abacus Concepts, Inc. USA) was used for the analysis. The level of significance was taken as p<0.05.

RESULTS

Body weight gain, food intake and feces

Figure 1 shows the body weight gains during the experimental diets. Figures 2 and 3 show the food intake and feces of the 12th week after the start of the experimental diet. The body weight gains of the control rats (C group) were approximately 300 g during administration of the experimental diets, with those of the control rats fed Maitake diets (CM group) showing a lower tendency than the C group. This tendency appeared immediately after the start of the experimental diets. Food intake of the C group was 21.60±5.45 g, while that of non-diabetic rats fed Maitake (CM group, 30.61±4.5 g) was significantly higher than that of the C group. Feces of the C group amounted to 2.99±0.42 g, while those of non-diabetic rats, fed Maitake (CM group, 5.86±1.04 g) increased significantly in comparison with that of the C group. Feces of the C group were significantly less than levels those of the other groups. During administration of the experimental diets, the body weight gain of the D group was approximately 70 g. The body weight gain of the diabetic rats fed Maitake (DM group) was lower than that of the C group but was significantly higher than that of the D group. Food intake of D group (33.06±7.31 g) was greater than that of C group. The food intake of diabetic rats fed Maitake (DM group, 24.72±5.31 g) was significantly lower than that of the D group. Feces of the D group came to 4.45±1.10 g, and those of diabetic rats, fed Maitake (DM group, 5.14±1.28 g) were slightly higher.

Glucose tolerance test and plasma insulin concentration

Figure 4 shows the glucose tolerance test of D and DM groups before administration of the experimental diets (8 wk old). Figure 5 shows the glucose tolerance test of all groups at the 10th week after the start of the experimental diets. The maximum glucose concentra-
Fig. 4. Blood glucose tolerance in rats before administration of experimental diets. The values are expressed as the mean±SD.

Fig. 5. Blood glucose tolerance in rats at 10th week of experimental diet administration. The values are expressed as the mean±SD, a: significantly different for D in comparison with C and for DM in comparison with CM by Scheffe’s test (p<0.05). b: significantly different for CM in comparison with C and for DM in comparison with D by Scheffe’s test (p<0.05). c: significantly different for DM in comparison with C by Scheffe’s test (p<0.05).

The fasting blood sugar at the 10th week after the start of the experimental diets in the C group was 131±19 mg/dL and 119±12 mg/dL in the CM group, with no difference between the two groups. Maximum glucose concentration in the C group was 204±19 mg/dL and 186±10 mg/dL in the CM group. When Maitake was administered to the control group, no effect was noted on the blood glucose. On the other hand, the blood glucose value in the D group was 225±49 mg/dL at fasting and the maximum blood glucose was 419±55 mg/dL at 60 min after injection of glucose. The blood glucose of the D group was significantly increased in comparison with the control groups. The blood glucose value in the DM group that was administered the experimental diet containing Maitake was 170±23 mg/dL at fasting and the maximum blood glucose was 250±41 mg/dL at 15 min after injection of glucose. The blood glucose of the DM group was significantly decreased in comparison with the D group. Figure 6 shows plasma insulin concentrations at the 10th week after the start of administration of experimental diets. The fasting insulin concentration in the C group was 21±8 μU/mL and that in the CM group was 20±14 μU/mL. Both groups showed very similar results. The plasma insulin concentration of the D group (6±7 μU/mL) was significantly lower than the C group at fasting. The plasma insulin concentration of the DM group (10±3 μU/mL) was similar to that of the D group. The plasma insulin concentration of the C group was 78±7 μU/mL at 15 min after injection of glucose and that of the CM group was 72±14 μU/mL. The difference value of the C group was 57±3 μU/mL and that of the CM group was 52±6 μU/mL. On the other hand, the plasma insulin concentration of the D group was 15±7 μU/mL at 15 min from the injection of glucose and the DM group was 41±16 μU/mL. The DM group was significantly higher than the D group. The difference value of the D group was 9±2 μU/mL and that of the DM group was 31±14 μU/mL. As the insulin concentration of the DM group significantly increased, the difference value of the DM group showed almost the same secretion as the C and CM groups. In short, when rats of the D group were injected with glucose solution, the insulin concentration rose slightly. When rats of the DM group were injected with glucose solution, insulin concentration at fasting had a low value. But the insulin concentration at 15 min after glucose injection in the DM group was the same as the insulin secretion from the control groups.

Islets of Langerhans

Figure 7 presents light microscope images of Langerhans' cells. In order to observe insulin secretion
In the islets of Langerhans, insulin was stained by the ABC method. Figure 7 (a), 7 (b), 7 (c) and 7 (d) are photographs of the C, CM, D and DM groups, respectively. These photographs are typical examples of the islets of Langerhans. Islets of the CM group were globular in form and showed a rich insulin stain. This was similar to the C group, but the Langerhans' cells of the D group were slightly smaller and less definite and showed a little insulin stain. Langerhans' cells of the DM group fed Maitake were small but retained their globular form and displayed recovered insulin stain. The exocrine section did not show any difference among the four groups.

*Serum composition*

Table 3 shows the serum composition when the rats were vivisected.

In the diabetes groups (D and DM groups), the blood glucose level was significantly higher than that of the non-diabetes groups (C and CM groups), with that of the D group being significantly higher than the DM group. In the case of fructosamine, diabetes groups (D and DM groups) showed significantly higher levels than
non-diabetes groups (C and CM groups), with the D group being significantly higher than the DM group. For 1.5-A.G. (1.5-anhydro-glucitol), the diabetes groups (D and DM groups) had significantly lower levels than the non-diabetes groups (C and CM groups), and the D group was significantly lower than the DM group.

**DISCUSSION**

When normal rats were fed the experimental diets including Maitake, their body weight gain was suppressed (Fig. 1) in spite of an increased food intake (Fig. 2). This had also been observed in our previous study (13) and may indicate a weight-reducing effect of Maitake (8). If there is such an effect, one possible reason might be the high fiber content of Maitake, which results in an increase in the volume of feces eliminated by the rats fed Maitake (Fig. 3). Future studies on this phenomenon are awaited.

When diabetic rats were given experimental diets including Maitake, a remarkable decrease in blood glucose was observed (13). In this study, we observed a change in the insulin concentration by glucose loading to STZ-induced diabetic rats. In contrast, when normal rats were injected with glucose, the plasma insulin concentration was not affected by Maitake administration (Fig. 6).

When rats of the D group were injected with glucose solution, the plasma insulin concentration rose only slightly. Immediately after the rats of the DM group were injected with glucose solution, their plasma insulin concentration was higher than that of the D group. When the rats were fed fungi such as Maitake, the abundant fiber volume could which can suppress glucose absorption from the intestines and prevent rises in blood glucose. As a result of this suppressed absorption, this phenomenon might improve blood glucose tolerance. However, when we administered glucose into the venter of rats, glucose tolerance was not improved. Furthermore, in the DM group the plasma insulin concentration rose on glucose loading, and insulin was higher in the diabetic rat group fed Maitake than in the diabetic rat group fed control diets. Eventually, we made the following hypothesis: As a result of stimulation of the β-cell by a substance included in Maitake, plasma insulin concentration was regained and the glucose level was suppressed. However, Maitake did not seem to influence the normal blood glucose level, insulin concentration or morphology β-cells in normal rats. In short, normal rats fed Maitake showed no signs of any effect other than the change in blood glucose.

Phlorizin increases the glucose discharge in the kidney, thereby normalizing the blood glucose level. In this case, phlorizin does not change the insulin level. However, in this study, the insulin levels were different in the D and DM group. The excreted glucosuria volumes of the groups were 24,500±11,000 mg/d (D group), 144±26 mg/d (DM group), 7±2 mg/d (C group), and 8±4 mg/d (CM group) (13). In short, in this case, we understood that the blood glucose was not decreased due to loss of glucose into urine. Maitake does not have the same function as phlorizin.

We guess that Maitake inhibits glucose absorption in the same manner as vogribose, and if this conjecture is correct, it would mean that Maitake has a lot of dietary fiber. However, when we studied the dietary fiber in Maitake in our previous study, we found no change in the body-weight-reducing effect (17). And in this study, there was no marked difference between the D group and DM group in feces volume. A vogribose-like effect may be at work in Maitake, but only a very weak one.

As another potential cause, Maitake normalization of blood glucose level may be attributable to reducing glucose absorption from the small intestine, resulting in regeneration of insulin secretion from β-cells. Leahy et al. have shown that a reduction in the ambient glucose level led to a recovery of glucose-induced insulin secretion (18). However, because the influence of Maitake on glucose absorption remains controversial, whether this possibility is involved needs further investigation. In future experiments, we want to measure the glucose absorption of rats fed Maitake.

We observed that Maitake prompted insulin secretion and decreased the blood glucose level in rats. These phenomena are known to occur with ganoderan of *Ganoderma lucidum* (10, 11). By i.p. administration to normal mice, ganoderan mediated the hypoglycemic effect. I.p. administration of ganoderan to alloxan-hyperglycemic mice also reduced the plasma glucose level.

Maitake also seems to possess anti-diabetic activities such as the reduction of blood glucose. Kubo et al. also reported that Maitake has anti-diabetic activity that is effective in KK-Ay mouse, a model of non-insulin-dependent diabetes mellitus (12). Further work will be needed to clarify the mechanism.

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